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Patentanmeldung Nr. Patent application No. Demande de brevet n°

02080019.9

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Anmeldung Nr.:
Application no.: 02080019.9 /
Demande no:

Anmeldetag:
Date of filing: 29.11.02 ✓
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

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Method for preparing a thickening agent, thickening agent thus prepared, use thereof and products containing the agent

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)
revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A23L1/00

Am Anmeldetag benannte Vertragsstaaten/Contracting states designated at date of
filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR

METHOD FOR PREPARING A THICKENING AGENT, THICKENING
AGENT THUS PREPARED, USE THEREOF AND
PRODUCTS CONTAINING THE AGENT

The invention relates to a method for preparing a thickening agent. The invention further relates to thickening agents thus prepared, to the use thereof in various products and to the products comprising the thickening agent.

- 5 Thickeners are used in a wide variety of applications, both in food and in non-food products. For food applications, thickeners can be roughly divided into two groups, polysaccharides and proteins. Examples of the first group are e.g. guar gum, xanthan gum, locust bean gum.
- 10 Examples of the second group are e.g. milk proteins. Among the milk proteins, whey proteins are widely used as ingredients in food products for their ability to form gels. β -Lactoglobulin is the major protein component of the whey protein from milk. It is a globular protein with a molar mass
- 15 of 18.3 kDa and a diameter of about 2 nm. When the protein is dissolved in an aqueous solution and heated above the denaturation temperature (about 78°C) it forms a gel. The globular structure unfolds at least partially and aggregates are formed. The gel is formed by heat treatment if the
- 20 concentration of the protein is above a critical value (C_p), and an appropriate ionic strength is applied.

- Polysaccharides have the advantage that they are effective thickeners in food products, even in low amounts. However, the price of these hydrocolloids is normally high.
- 25 Moreover, at elevated concentrations they may often give rise to taste defects. When used in dairy products like desserts, they are considered non-natural.

Proteins are normally less effective (on a w/w basis) in thickening compared to hydrocolloids. Thus, even though

their price may be considerably lower than for hydrocolloids, the higher dose required abolishes the price advantage.

As explained above, globular proteins form a gel when heated at neutral pH (around 7). However, the concentration
5 needed to form the gel is relatively high, e.g. more than 5% (w/w). Moreover, a gel thus obtained is irreversibly formed and is therefore not suitable for use as thickener in a range of products. The gel would have to be dried and/or comminuted thus losing its thickening capacity. On the other hand, if
10 whey proteins are thermally modified at neutral pH and low concentrations to avoid the undesired gel formation, the thickening capacity is very poor or not present at all.

There is thus a need for proteins that are highly effective in thickening at low concentrations.

15 In the research that led to the present invention it was found for β -lactoglobulin that the structures obtained at low pH confer to the solution containing them a much higher viscosity and have thus a higher gelling capacity than the structures formed by heating β -lactoglobulin at pH 7. Gelling
20 agents of such low pH are however not practically useful.

When a solution of β -lactoglobulin is heated at a pH of about 2, denaturation leads to a different type of aggregation than at neutral pH. This low-pH denaturation leads to protein aggregates which are joined by physical
25 forces, whereas denaturation at a pH around 7 or higher will lead to aggregates which are covalently bound through disulfide bonds.

It was found that heating a solution of β -lactoglobulin at a pH around 2 leads to formation of
30 fibrillar protein structures. As stated above, it is generally accepted that these fibrils are constituted by aggregates held together by physical forces. The skilled

person would expect that fibrils thus formed would decompose again upon pH increase.

In the research that led to the present invention it was surprisingly found that these fibrils are irreversibly
5 formed when the heating time at or above denaturation temperature is longer than 10 minutes.

The invention thus relates to a method comprising the steps of:

a) heating a solution comprising one or more
10 substantially non-denatured globular proteins at a temperature at or above denaturation temperature, preferably between 50 and 100°C and a pH below 3.0; and

b) performing one or more of the following steps in random order:

- 15 i) increasing the pH;
- ii) increasing the salt concentration;
- iii) concentrating the solution.

In solutions containing globular proteins that are treated in this way, fibrils are formed having an
20 unexpectedly high gelling and/or thickening capacity. The fibrils are irreversibly formed and can be used at any desired pH or ionic strength.

Heating the solution is preferably performed during at least 10 minutes, preferably at least 1 hour, more
25 preferably at least 6 hours, most preferably at least 8 hours.

The pH of treatment is preferably below 2.8, preferably below 2.5, more preferably below 2.2.

The total heating time required to obtain the effect
30 may be achieved by batch wise heating, continuous flow heating or a combination of subsequent heating steps, e.g. by means of circulating a solution through a heating system.

Optionally, the solution is cooled before performing one or more of steps i) to iii).

It is preferred to cool the solution to a temperature between the denaturation temperature and 20°C, preferably
5 between the denaturation temperature and 5°C.

When the pH is increased this is preferably to a value between 3.9 and 9, preferably to about neutral pH. Most food applications have a neutral, near neutral or slightly acid pH.

10 Advantageously, the salt concentration is increased to a maximum of 0.2 M, preferably to 0.1 M. The salt used for increasing the salt concentration is preferably the salt of a divalent ion, preferably calcium. It was found that by adding calcium the gelling capacity is further increased.

15 According to a preferred embodiment step i) is performed prior to step ii) because pH adjustment in dilute systems is easier to carry out.

In order to obtain a dry product which is more stable upon storage the method further comprises the step of drying
20 the solution to obtain a dry product. It was found that upon reconstituting the thickening agent from the powder obtained after drying the same or similar gelling properties were obtained. It is practical when the drying comprises spray drying. The dry product is preferably a powder. Alternatively
25 granulates can be envisaged.

The method of the present invention can be performed with a wide variety of globular proteins, such as whey proteins, egg albumins, blood globulins, soya proteins, wheat proteins, potato proteins or pea proteins. In a preferred
30 embodiment, the globular protein is a whey protein isolate or a whey concentrate, preferably a whey protein concentrate enriched in (e.g. > 40%) β -lactoglobulin. In a much preferred embodiment the globular protein is β -lactoglobulin.

The invention further relates to a thickening agent based on a system of one or more proteins that are aggregated to form fibrils, characterized in that the thickening agent has a higher viscosity than a similar thickening agent based on a system of the same one or more proteins in the same concentration in which the proteins are not aggregated into fibrils. Fibrils in this respect are preferably fibrils consisting of protein and having an aspect ratio of 5 or higher. The aspect ratio is the ratio between length and width or length and height or length and diameter. The length of the fibrils is preferably equal to or above 100Å and equal to or below 1 mm, preferably below 100 µm. These fibrils can be made visible by means of a microscope. Figure 1 shows the fibrils through a microscope at neutral pH. From this it follows that the fibrils are stable.

The above described thickening agent can be obtained by the method of the invention or by any other means that leads to the above described structural properties.

The thickening agent of the invention can be used as a stabilizer of foams, dispersions and emulsions. Foams are systems of a gas in a liquid. Emulsions are liquids in liquids and dispersions are solids in liquids. Usually these systems cannot exist without the help of thickening agent that helps in maintaining the disperse phase uniformly distributed in the continuous phase. The thickening agent of the invention was found to be very suitable for this purpose.

The thickening agent can be used in food stuffs, such as dairy products. When using β-lactoglobulin, whey protein concentrate or whey protein isolate as the globular protein that constitutes the thickening agent the product obtained can be an all milk product.

Whey protein concentrates normally comprise 25-90% (w/w) whey protein. Whey protein isolates usually comprise

> 90% whey protein.

The thickening agent can also be used in meat products, e.g. comminuted meat products (Frankfurter sausages), hamburgers, luncheon meat, p  te's, poultry, fish
5 meat products or meat replacers on vegetable basis, to enhance the water-binding and/or texture of the product.

Alternative applications of the thickening agent of the invention can be found in non-food products such as paints, cosmetics, toothpastes, deodorants etc.

10 The invention further relates to products comprising the thickening agent of the invention, such as food stuffs, in particular dairy products or meat products, but also non-food products, e.g. paints, cosmetics, toothpastes, deodorants.

15 According to a further aspect thereof the invention relates to a protein composition comprising one or more particles having texturizing properties, wherein the protein molecules are aggregated into fibrils. Texturizing properties comprise the ability to promote or modify the viscosity or
20 gelling ability of a product containing the composition. Preferably, the fibrils have an aspect ratio, which is defined as the ratio between length and width or length and height or length and diameter, of 5 or higher. The length of the fibrils is preferably equal to or above 100   and equal to
25 or below 1 mm, preferably below 100   m.

The present invention will be further illustrated in the examples that follow and that are not intended to limit the invention.

EXAMPLESEXAMPLE 1

Preparation of β -lactoglobulin gels according to the
invention, and determination of critical gelling
5 concentration

β -Lactoglobulin (β -lg) was obtained from Sigma (L-0130) and is a mixture of the genetic variants A and B. The protein was dissolved (3% w/w) in a HCl solution at pH 2. To remove traces of calcium ions from the β -lg, and to obtain a
10 protein solution with the same pH and ionic strength as the solvent, the protein was diluted repeatedly with HCl solvent and filtered through a 3K filter in an Omegacell™ membrane cell (Filtron) at 4°C and a maximum pressure of 3 bar. The procedure was stopped, when the pH and conductivity of the
15 diluted solution and the solvent were the same.

The β -lg solution was centrifuged at 22600g for 30 min. To remove any traces of undissolved protein, the supernatant was filtered through a protein filter (FP 030/2, 0.45 mm, Schleicher & Schuell). A UV spectrophotometer was
20 used to determine the β -lg concentration at a wavelength of 278 nm.

β -Lactoglobulin (w/w) as prepared above diluted to a concentration of 2% was heated at 80°C for 10 h in a water bath. After cooling, the pH was adjusted to pH 7 or 8 with
25 0.1 and 1 M NaOH. Various CaCl₂ concentrations (0.005, 0.0075, 0.01, 0.05, and 0.1 M) were added very carefully on ice, and the solution was mixed well. After this procedure, the solution was poured into the VOR rheometer (Bohlin concentric cylinder geometry C14) to determine the critical
30 gelling concentration. The sample in the VOR was heated from 3°C to 25°C. After 3 h in rest, a strain sweep was performed (frequency 1 Hz, temperature 25°C, strain 0.000206-0.206).

The procedure was repeated for various protein concentrations. To determine the critical gelling concentration C_p , first the G' (the "elastic modulus", a characteristic for the elastic component of a system) was determined for various protein concentrations (in the linear region of the curve). A plot was made of $(G')^{1/t}$ versus concentration c , for t ranging between 1.7 and 4.5. t is the scaling factor. In the fitting procedure, we make use of the physical fact that extrapolation of $(G')^{1/t}$ to zero should yield the same C_p for all $t > 0$. The scaling assumption has the implication that when t is close to the correct value, the data points will be on a straight line. When t is larger than the correct value, the fit through the points will bend away from the straight line and will lie below it. When t is smaller than the correct value, the fit through the points will also bend away from the straight line but will now lie above it. In that case, the slope of the fit at the intercept with the horizontal axis will be zero. Therefore, in the fitting procedure we use the fact that the curvature of the fit will change if different values for t are chosen, while the intercept C_p will have to remain the same.

C_p was determined from fits through the data points that are closest to a straight line in determining an average intercept, C_p . It appeared that the C_p values for the protein system according to the invention were considerably lower than for the reference (not-modified) protein system. (see examples)

The results of this experiment are shown in Table

1.

EXAMPLE 2Preparation of β -lg gels according to the conventional (neutral pH) gelation method, and determination of the critical gelling concentration

5 β -Lactoglobulin (β -lg) was obtained from Sigma (L-0130) and is a mixture of the genetic variants A and B. The protein was dissolved (3% w/w) in a HCl solution at pH 2. To remove traces of calcium ions from the β -lg, and to obtain a protein solution with the same pH and ionic strength as the
10 solvent, the protein was diluted repeatedly with HCl solvent and filtered through a 3K filter in an Omegacell™ membrane cell (Filtron) at 4°C and a maximum pressure of 3 bar. The procedure was stopped, when the pH and conductivity of the diluted solution and the solvent were the same.

15 The β -lg solution was centrifuged at 22600g for 30 min. To remove any traces of undissolved protein, the supernatant was filtered through a protein filter (FP 030/2, 0.45 mm, Schleicher & Schuell). A UV spectrophotometer was used to determine the β -lg concentration at a wavelength of
20 278 nm.

3% β -lg samples at pH 7 or 8 were heated at 80°C for 30 min. After cooling, 0.01 M CaCl₂ was added very carefully on ice, and the solution was mixed well. After this procedure, the solution was poured in the VOR (Bohlin
25 concentric cylinder geometry C14). The sample in the VOR was heated from 3°C to 25°C. After 3 h in rest, a strain sweep was performed (frequency 1 Hz, temperature 25°C, strain 0.000206-0.206). Subsequently the critical gelling concentration of the conventionally formed β -lactoglobulin
30 gel was measured. The results are shown in Table 1.

Table 1

Determination of the Critical gelling concentration
(gels prepared according to examples 1 and 2)

Heating conditions	Example no.:	Final pH	[mM] CaCl ₂	Critical gelling concentration (% w/w)
pH 2, 10 hrs, 80°C	1	7.0	0	1.3
pH 2, 10 hrs, 80°C	1	7.0	5	1.1
pH 2, 10 hrs, 80°C	1	7.0	7.5	1.0
pH 2, 10 hrs, 80°C	1	7.0	10	0.1
pH 2, 10 hrs, 80°C	1	7.0	50	0.6
pH 2, 10 hrs, 80°C	1	7.0	100	0.7
pH 2, 10 hrs, 80°C	1	8.0	10	0.4
pH 2, 10 hrs, 80°C	1	8.0	50	0.6
pH 2, 10 hrs, 80°C	1	8.0	100	0.9
pH 7, 0.5 hrs, 80°C	2	7.0	10	No gel formed at 3%
pH 7, 0.5 hrs, 80°C	2	8.0	10	No gel formed at 3%

The results show that β -lactoglobulin modified by the acid pretreatment has a higher gelling ability than β -lactoglobulin which is not acid-modified.

5 EXAMPLE 3

Modification of BiprotTM

BiprotTM, a whey protein isolate powder (95% protein, w/w), was obtained from Davisco, USA. Besides β -lactoglobulin, BiprotTM also contains α -lactalbumin, bovine serum albumin and immunoglobulines.

Modification of BiprotTM was carried out as follows: Four BiprotTM solutions were prepared in demineralised water in concentrations of 3, 4, 5 and 6% w/w. The pH was adjusted to pH 2, using HCl. The solutions were heated for 10 hours at 80°C. After cooling, the samples were neutralised with NaOH to pH 7, and cooled further to 3°C, after which CaCl₂ (5 mM) was added to half of the samples. After 3 hours, all samples were assessed visually. The results are given in table 2.

A control experiment was carried out in the following way. Bipro™ solutions in demineralized water were made (3, 4, 5, 6% w/w) having a pH of 7. The solutions were heated at 80°C for 10 hrs, then cooled to 3°C and CaCl₂ was added to half of the samples. After 3 hours, the samples were assessed visually. The results are shown in table 2.

Table 2

Visual rheological properties of modified and not-modified Bipro™ (from example 3)

Bipro™ samples:	no CaCl ₂ added	5 mM CaCl ₂ added
pH 2 modified and neutralised: (% w/w)		
3	Viscous solution	Gel
4	Very viscous sol.	Gel
5	Very viscous sol.	Firm gel
6	Very viscous sol.	Very firm gel
Not modified:		
3	Low viscous liquid	Liquid
4	Low viscous liquid	Liquid
5	Low viscous liquid	Liquid
6	Low viscous liquid	Liquid

From the table it clearly follows that treatment according to the invention, of a whey protein product comprising different types of protein, also leads to strongly enhanced gelling capacity and a strong increase in viscosity.

EXAMPLE 4

Custard-like cream dessert

Modified Bipro™ was obtained as described in Example 3 by freeze-drying a sufficient amount of the neutralized 5% solution. The powder thus obtained can be used

directly in the applications below, or mixed with calcium chloride prior to use in the applications.

Composition:

	A. traditional	B. invention
	(grams)	(grams)
5		
Skim milk	355	355
Cream (40% fat)	65	65
10 Water	444	444
Protein:		
Esprion 300U	10	-
(DMV International)		
Modified Bipro™	-	0.8
15 Saccharose	60	60
Lactose	28	37
Modified starch	38	38
(C*tex 06201 from Cerestar)		
Carrageenan	0.3	0.3
20 (CL 360C, Danisco)		
Flavouring	q.s.	q.s.
(e.g. vanilla)		
Colouring	q.s.	q.s.

25 Esprion™ 300U is a whey protein concentrate having 30% protein (w/w).

All the ingredients were mixed in the cold milk (approx. 7°C), and left to hydrate for 20 minutes at a temperature < 10°C. The mixture was heated for 10-20 seconds at 140°C using an UHT pasteuriser (APV, Denmark) fitted with a holding tube, and subsequently cooled to < 10°C, and packaged. Storage was at a temperature below 10°C.

Products obtained are tested by a panel, and a

texture measurement was carried out using the Stevens Texture Analyser™ (Stevens Instruments, UK) equipped with a disc probe. The resistance of the probe was measured as the probe penetrates the sample within a determined period of time over
5 a specified distance.

The test results showed that, despite the low dosage of modified Bipro™, the texture of Sample B was much better (better mouth feel, higher viscosity) than sample A.

10 EXAMPLE 5

Application in drinking yogurt

Yogurt A (reference) was prepared as follows.
117 grams of Espriion™ 300U was dissolved in 1 liter of water. 280 Grams of this solution was mixed with 720 grams of
15 skim milk. The final protein concentration of this solution was 3.5% (w/w). The solution was heated to 65°C and homogenised at this temperature, after which it was pasteurised for 6 minutes at 92°C. The pasteurised milk was cooled to 32°C, and inoculated with a yogurt culture (0.02 %
20 Yoflex™ 380 from Chr. Hansen). Fermentation was continued for approx. 14-16 hours until a pH of 4.2-4.3 was reached.

Drinking yogurt was prepared by blending the freshly prepared yogurt with a fruit preparation (25% water, 25% fruit juice, 50% sugar obtainable from Wild, Germany) in
25 a ratio 80% yogurt/20% fruit preparation. Before adding to the yogurt, the fruit preparation was pasteurised at 85°C for 5 minutes and cooled to 20°C.

The mixture of yogurt and fruit preparation was subjected to a low-pressure homogenisation at 1-3 MPa.
30 The drinking yogurt was then cooled to < 10°C, packaged and stored below 10°C.

Yogurt B including the protein preparation according to the invention was prepared in a similar way as A

but the starting milk was composed of 280 grams of an 0.8% (w/w) solution of modified Bipro™ (from the same source as example 4; protein content = 90% w/w) and 10.9% lactose was mixed with 720 grams of skim milk. The final protein

5 concentration of this solution was 2.7%

Drinking yogurt was prepared in a comparable way as described for (drinking) yogurt A.

Despite the lower protein concentration in drinking yogurt B, the product obtained had a higher viscosity than
10 the reference drinking yogurt A. A test panel evaluation resulted in a preference for the drinking yogurt B, based on a more pleasant mouth feel.

CLAIMS

1. Method for the preparation of a thickening agent, comprising the steps of:

a) heating a solution comprising one or more substantially non-denatured globular proteins capable of being thermally denatured at a temperature at or above the denaturation temperature of the protein, preferably between 50 and 100°C, and a pH below 3.0; and

b) performing one or more of the following steps in random order:

- 1) increasing the pH;
- ii) increasing the salt concentration;
- iii) concentrating the solution.

2. Method as claimed in claim 1, wherein the solution is heated during a period of at least 10 minutes, preferably at least 1 hour, more preferably at least 6 hours, even more preferably at least 8 hours.

3. Method as claimed in any one of the claims 1 and 2, wherein the solution is cooled before performing one or more of steps i) to iii).

4. Method as claimed in claim 3, wherein the solution is cooled to a temperature between denaturation temperature and 20°C, preferably between denaturation temperature and 5°C.

5. Method as claimed in any one of the claims 1-4, wherein the heating is performed at a pH below 2.8, preferably below 2.5, more preferably below 2.2.

6. Method as claimed in any one of the claims 1-5, wherein the pH is increased to a value between 3.9 and 9, preferably to about neutral pH.

7. Method as claimed in any one of the claims 1-6, wherein the salt concentration is increased to a maximum of

0.2M, preferably to 0.1M.

8. Method as claimed in any one of the claims 1-7, wherein the salt used for increasing the salt concentration is the salt of a divalent ion, preferably calcium.

5 9. Method as claimed in any one of the claims 1-8, wherein step i) is performed prior to step ii).

10. Method as claimed in any one of the claims 1-9, further comprising the step of drying the solution to obtain a dry product.

10 11. Method as claimed in claim 10, wherein the drying comprises spray drying.

12. Method as claimed in any one of claims 10-11, wherein the dry product is a powder.

15 13. Method as claimed in claim 1-12, wherein the globular protein is selected from the group consisting of whey proteins, egg albumins, blood globulins, soya proteins, wheat proteins, potato proteins, pea proteins.

20 14. Method as claimed in claim 13, wherein the globular protein is a whey protein isolate, a whey protein concentrate, and preferably a whey protein concentrate enriched in β -lactoglobulin.

15. Method as claimed in any one of the claims 13 and 14, wherein the globular protein is β -lactoglobulin.

25 16. Thickening agent based on a system of one or more proteins that are aggregated to form fibrils, characterized in that the thickening agent has a higher viscosity than a similar thickening agent based on a system of the same one or more proteins in the same concentration in which the proteins are not aggregated into fibrils.

30 17. Thickening agent obtainable by the method as claimed in any one of the claims 1-15,

18. Thickening agent as claimed in claim 16 obtainable by the method as claimed in any one of the claims

17

1-15.

19. Thickening agent as claimed in claim 16 in dry form obtainable by the method as claimed in any one of the claims 10-12.

5 20. Thickening agent as claimed in any one of the claims 16-19 for use as a stabilizer of foams, dispersions and emulsions.

21. Thickening agent as claimed in any one of the claims 16-19 for use in dairy products.

10 22. Thickening agent as claimed in any one of the claims 16-19 for use in meat products.

23. Thickening agent as claimed in any one of the claims 16-19 for use in paints.

15 24. Thickening agent as claimed in any one of the claims 16-19 for use in toothpastes, cosmetics, deodorants.

25. Dairy product comprising the thickening agent as claimed in any one of the claims 16-19.

26. Meat product comprising the thickening agent as claimed in any one of the claims 16-19.

20 27. Paint comprising the thickening agent as claimed in any one of the claims 16-19.

28. Toothpaste comprising the thickening agent as claimed in any one of the claims 16-19.

25 29. Cosmetic comprising the thickening agent as claimed in any one of the claims 16-19.

30. Deodorant comprising the thickening agent as claimed in any one of the claims 16-19.

31. Protein composition comprising one or more particles having texturizing properties, wherein the protein molecules are aggregated into fibrils.

32. Protein composition as claimed in claim 31, wherein the texturizing properties comprise the ability to promote or modify the viscosity or gelling ability of a

18

product containing the composition.

33. Protein composition as claimed in any one of the claims 31 and 32, wherein the fibrils have an aspect ratio, which is defined as the ratio between length and width or length and height or length and diameter, of 5 or higher.

34. Protein composition as claimed in any one of the claims 31-33, wherein the length of the fibrils is preferably equal to or above 100Å and equal to or below 1 mm, preferably below 100 µm.

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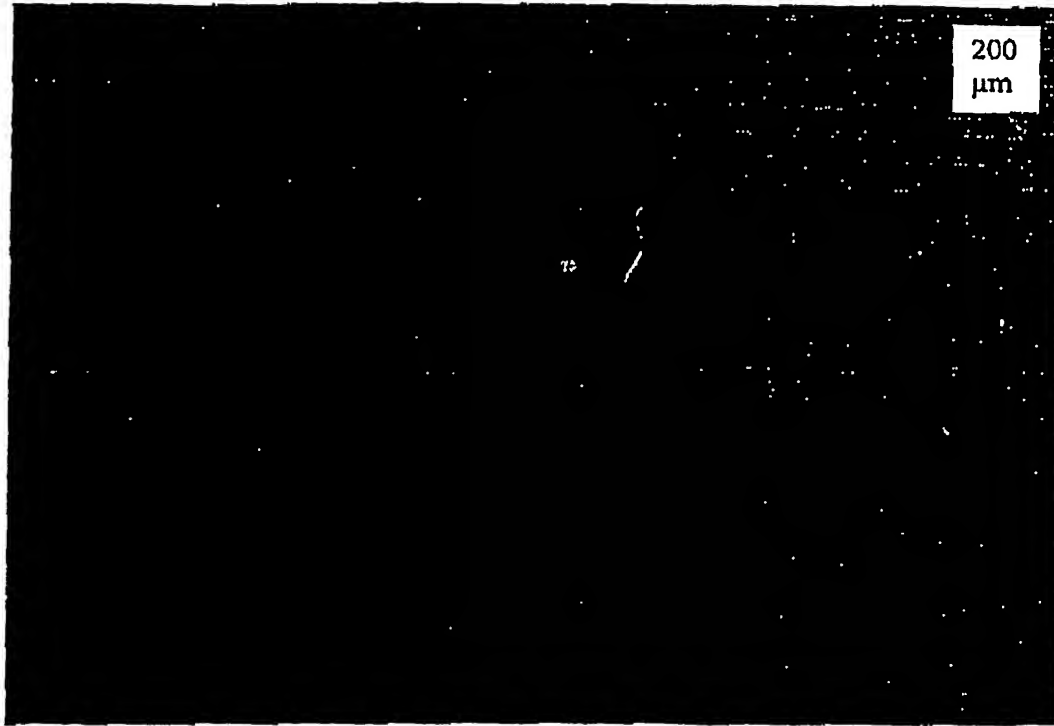
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ABSTRACT

The invention relates to a method for the
5 preparation of a thickening agent, comprising the steps of
heating a solution comprising one or more substantially non-
denatured globular proteins capable of being thermally
denatured at a temperature at or above the denaturation
temperature of the protein, preferably between 50 and 100°C,
10 and a pH below 3.0; and performing one or more of the
following steps in random order: increasing the pH;
increasing the salt concentration; and concentrating the
solution. Preferably, the solution is heated during a period
of at least 10 minutes, preferably at least 1 hour, more
15 preferably at least 6 hours, even more preferably at least 8
hours. The invention also relates to the thickening agent
thus obtained, to the use thereof in food and non-food
applications and to the food and non-food products containing
the thickening agent.

1/1



polarised light

Figure 1

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